# Region-Dependent and Stage-Specific Effects of Stress, Environmental Enrichment, and Antidepressant Treatment on Hippocampal Neurogenesis

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**ABSTRACT: Chronic stress and depression are associated with decreased levels of hippocampal neurogenesis. On the other hand, antidepressants as well as environmental enrichment may rely in part on their pro-neurogenic effects to improve cognition and mood. Because a functional heterogeneity has been consistently reported along the septo-temporal axis of the hippocampus, regional changes in neurogenesis could differentially contribute to these effects and affect distinct hippocampal functions. Mapping these regional changes could therefore provide a better understanding of the function of newborn neurons. While some studies report region-specific effects of stress and antidepressants on neurogenesis, it is unclear whether these changes affect distinct populations of newborn neurons according to their developmental stage in a region-specific manner. By using endogenous markers and BrdU labeling we quantified the regional changes in cell proliferation and survival as well as in the number of neuronal progenitors and immature neurons following unpredictable chronic mild stress (UCMS), environmental enrichment (EE) and chronic fluoxetine (20 mg/kg/day) treatment along the septo-temporal axis of the hippocampus. EE promoted cell proliferation and survival of 4-week-old newborn cells as well as increased the number and proportion of post-mitotic immature neurons specifically within the septal hippocampus. By contrast, UCMS uniformly decreased cell proliferation, survival and immature newborn neurons but differentially affected progenitor cells with a decrease restricted to the temporal regions of the hippocampus. Whereas fluoxetine treatment in control mice affected proliferation and survival specifically in the temporal hippocampus, it reversed most of the UCMS-induced alterations all along the septo-temporal axis. These results highlight that different factors known for exerting a mood improving effect differentially regulate neurogenesis along the septo-temporal axis of the hippocampus. Such region and stage specific effects may correlate to distinct functional properties of newborn neurons along the septo-temporal axis of the hippocampus which may contribute differently to the pathophysiology of affective disorders.** **© 2013 Wiley Periodicals, Inc.**

**KEY WORDS: hippocampal neurogenesis; septo-temporal axis; chronic stress; environmental enrichment; depression; antidepressants**

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## INTRODUCTION

The hippocampus is a curve shaped structure that extends in rodents both rostro-caudally and dorso-ventrally from the septal nuclei of the basal forebrain to the temporal lobe. Along this septo-temporal axis striking variations have been described in term of connectivity (Swanson and Cowan, 1977; Amaral and Witter, 1989), gene expression (Thompson et al., 2008; Fanselow and Dong, 2010), neurotransmission (Gage and Thompson, 1980; Jinno and Kosaka, 2006, 2010) and synaptic plasticity (Maggio and Segal, 2007, 2009) which altogether might explain the functional heterogeneity of this structure (Moser and Moser, 1998; Bannerman et al., 2004). While the septal pole seems more implicated in cognitive aspects of the hippocampal functions such as learning and memory (Moser et al., 1995), the temporal pole may underlie emotional aspects of hippocampus-driven functions and contribute in anxiety-related behaviors, motivational behaviors and regulation of the stress response (Henke, 1990; Kjelstrup et al., 2002; Herman and Mueller, 2006).

The hippocampus is known to be vulnerable to environmental challenges and displays an important ability to undergo plastic changes. In particular, the birth and functional integration of new neurons in the dentate gyrus of the hippocampus during adult life is highly modulated by intrinsic and environmental factors. Chronic exposure to stress and glucocorticoids severely impairs neurogenesis (Gould et al., 1992; Cameron and Gould, 1994; Rodriguez et al., 1998; Alonso et al., 2004; Wong and Herbert, 2006), and several studies now link impaired neurogenesis to mood and affective disorders (for a review, Petrik et al., 2012). On the other hand, mood-improving drugs, housing in an enriched environment and physical exercise are thought to modulate both cognitive and emotional behaviors in part by stimulating hippocampal neurogenesis (Bruel-Jungerman et al., 2005; Schloesser et al., 2010; Surget et al., 2011). However, given the aforementioned anatomical and functional hippocampal heterogeneity, it remains unclear whether these environment-related changes in neurogenesis occur differentially along the septo-temporal axis of

the hippocampus and/or affect different stages of neuronal development in a region-specific manner. This could be of particular relevance as gradients of activity and maturation of newborn neurons have already been described along the septotemporal axis of the hippocampus (Snyder et al., 2009, 2012; Piatti et al., 2011). Therefore mapping the regional changes in neurogenesis following environmental manipulations might be useful to better understand the role of adult-newborn neurons in hippocampal functions.

Considering its preferential involvement in emotional behaviors, it has been suggested that changes in neurogenesis following stress or antidepressant treatment might be more prominent in the temporal hippocampus. Several studies have now described such regional changes in stress- or depression-related paradigms but have yielded conflicting results. While some report similar impairments in cell proliferation, survival or in the number of new neurons in both septal and temporal divisions (Brummelte and Galea, 2010; Oomen et al., 2010; Païzanis et al., 2010; Rainer et al., 2011; Nollet et al., 2012), others observe detrimental effects of stress specifically in the temporal hippocampus (Jayatissa et al., 2006; Zuena et al., 2008; Brummelte and Galea, 2010; Oomen et al., 2010; Morley-Fletcher et al., 2011; Hawley and Leasure, 2012; Tanti et al., 2012) or even in the septal hippocampus (Ho and Wang, 2010; O'Leary et al., 2012). Similarly, studies investigating regional changes in neurogenesis following antidepressant treatment or mood-improving compounds have shown temporal specific effects (Banasr et al., 2006; Soumier et al., 2009; Païzanis et al., 2010; Mahar et al., 2011; Morley-Fletcher et al., 2011; Felice et al., 2012; O'Leary et al., 2012), septal specific effects (Jayatissa et al., 2006; Elizalde et al., 2010) or both (Jayatissa et al., 2006; Païzanis et al., 2010; Morley-Fletcher et al., 2011; Rainer et al., 2011; Nollet et al., 2012) depending on whether the effects of antidepressants were assessed in control/unchallenged or stressed animals. Inconsistencies in these previous reports highlight that more studies are needed to increase our knowledge about the regional changes in neurogenesis associated to environmental factors and antidepressant therapy.

Along with differences in species and gender of animals, paradigms, type of antidepressant, and anatomical boundaries used to define septal and temporal divisions, these discrepancies might result from the fact that in most of these studies the region-specific effects observed seem also to depend upon the developmental stage of newborn neurons that is addressed. Supporting this view, several studies show that the topographical effects of antidepressants or stress on proliferation do not correlate with the topographical changes observed in cell survival or in the number of newborn neurons (Banasr et al., 2006; Soumier et al., 2009; Oomen et al., 2010; Païzanis et al., 2010; Rainer et al., 2011; O'Leary et al., 2012). It is therefore possible that environmental factors and antidepressants have different stage-specific effects along the septo-temporal axis of the hippocampus which may not be identified by neuronal markers expressed during multiple stages of development.

To address this question, we used two paradigms known to reliably modulate neurogenesis: an environmental enrichment and the Unpredictable Chronic Mild Stress (UCMS) regimen with concomitant treatment with the Selective Serotonin Reuptake Inhibitor (SSRI) fluoxetine. Changes in cell survival and proliferation along the septo-temporal axis were respectively assessed using BrdU and Ki-67 labeling. In order to quantify distinct populations of newborn neurons according to their stage of maturation, we also performed triple labeling for Doublecortin (DCX), Prox-1 and Calretinin (CR). Since CR is only transiently expressed in immature neurons at post-mitotic stage (Brandt et al., 2003), whereas DCX is expressed in type 2b/3 neuronal progenitors but also in early post-mitotic immature neurons, this allowed us to quantify the number of neuronal progenitors (DCX+/Prox-1+/CR- cells), early immature neurons that reached a post-mitotic stage (DCX+/Prox-1+/CR+ cells), late post-mitotic newborn neurons expressing CR but not DCX (DCX-/Prox-1+/CR+ cells) as well as the proportion of cells that reached a post-mitotic stage as an index of maturation or stage-specific survival along the septo-temporal axis of the hippocampus (Fig. 1B). To allow a reliable topographical segregation of the hippocampus, these quantifications were performed in horizontal sections spread into five subdivisions along its septo-temporal axis.

Here we show that whereas UCMS and fluoxetine treatment affect both septal and temporal neurogenesis but in a stage-specific manner, enrichment stimulates neurogenesis and promotes the maturation of newborn neurons specifically in the septal hippocampus.

## MATERIAL AND METHODS

### Animals

Male BALB/cByJ mice aged 7 weeks obtained from the Centre d'Elevage Janvier (Le Genest Saint Isle, France) were used in this study. All animals were group-housed (4–5 per cage) and kept under standard conditions (12/12 h light–dark cycle –lights on at 9:00/off at 21:00–, 22 ± 1 °C, food and water ad libitum) in standard cages (42 cm × 27 cm × 16 cm) with shelter for one week prior to the start of the experiment. Animal care and treatment were all in accordance with the European Community Council directive 86/609/EEC.

### Unpredictable Chronic Mild Stress (UCMS) and Antidepressant Treatment

A first cohort of mice was used for the UCMS experiment (Fig. 1A). Mice were divided into four groups: Control-Vehicle, Control-Fluoxetine, UCMS-Vehicle, and UCMS-Fluoxetine ($n$ = 8 per group). UCMS-exposed mice were isolated in individual cages (24 cm × 11 cm × 12 cm) while nonstressed controls were kept group-housed in standard cages. The UCMS regimen used in our study is based on the Chronic

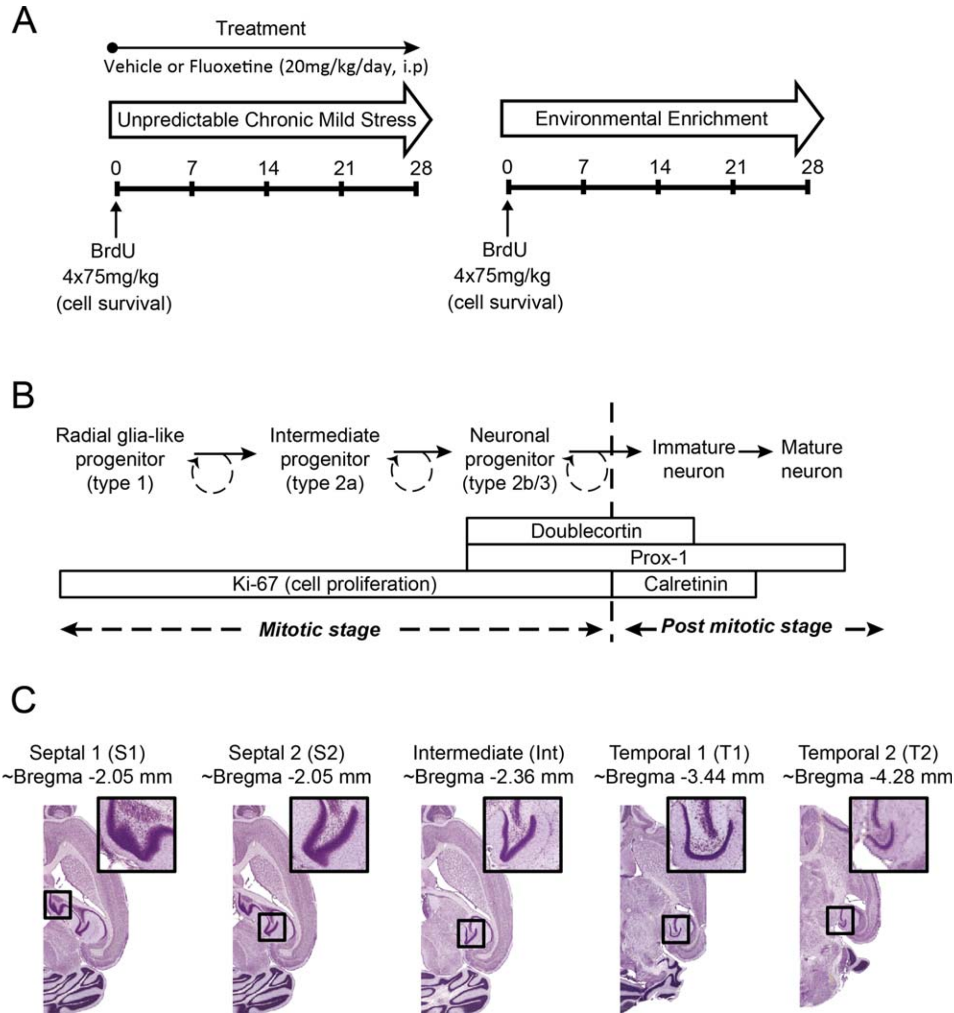


**FIGURE 1. Schematic representations of the experimental design, cell markers and topographical divisions of the hippocampus used in this study. A: Experimental design. Mice were either exposed to the Unpredictable Chronic Mild Stress (UCMS) model of depression or kept in standard conditions for 28 days. Both groups were either treated daily with fluoxetine (20 mg/kg/day, i.p) or vehicle (0.9% NaCl) until the end of the experiment. A second cohort of animals was divided into two groups in which mice were either housed in an enriched environment (EE) or kept in standard housing for 28 days. In both experiments BrdU injections (4 × 75 mg/kg) were made two days prior to the start of the experiment in order to assess cell survival. B: Schematic representation of the cell markers used in this experiment. At the end of both experiments animals were sacrificed and immunohistochemistry with Ki-67 was performed to quantify the number of proliferating cells. Triple labeling with doublecortin (DCX), Prox-1, and calretinin (CR) was used to quantify the density of neuronal progenitors (DCX+/Prox1+/CR− cells), early immature neurons that reached a post-mitotic stage (DCX+/Prox-1+/CR+ cells) and late immature neurons expressing CR but not DCX (DCX-/Prox1+/CR+ cells. C: Topographical divisions of the hippocampus along its septo-temporal axis used in both experiments to quantify the regional changes in neurogenesis induced by UCMS, EE, and fluoxetine treatment. Images and coordinates are adapted from Paxinos and Franklin's mouse brain atlas (Franklin and Paxinos, 2008). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]**

Mild Stress procedure developed in rats by (Willner et al., 1992) and adapted to mice from previous studies (Ducottet et al., 2003; Mineur et al., 2003; Santarelli et al., 2003; Ducottet and Belzung, 2004; Pothion et al., 2004).

Stressed mice were repeatedly exposed to various psychosocial stressors of mild intensity according to a semi-random schedule for four weeks. Stressors used consisted in successive sawdust changes, removal of sawdust, damping the sawdust, substitution of sawdust with water (21 °C), tilting the cages by 45°, placing a mouse into a cage that has been previously occupied by another mouse, restraint stress in small tubes for 1 h and changes in length or time of light/dark cycle.

Concomitant with the start of the UCMS procedure and until sacrifice all animals were treated daily (i.p, 10ml/kg) with either Fluoxetine hydrochloride (20 mg/kg prepared in saline; Sequoia Research Products) or Vehicle (saline). This dose was previously shown to be able to counteract the behavioral and physiological impairments induced by UCMS (Surget et al.,

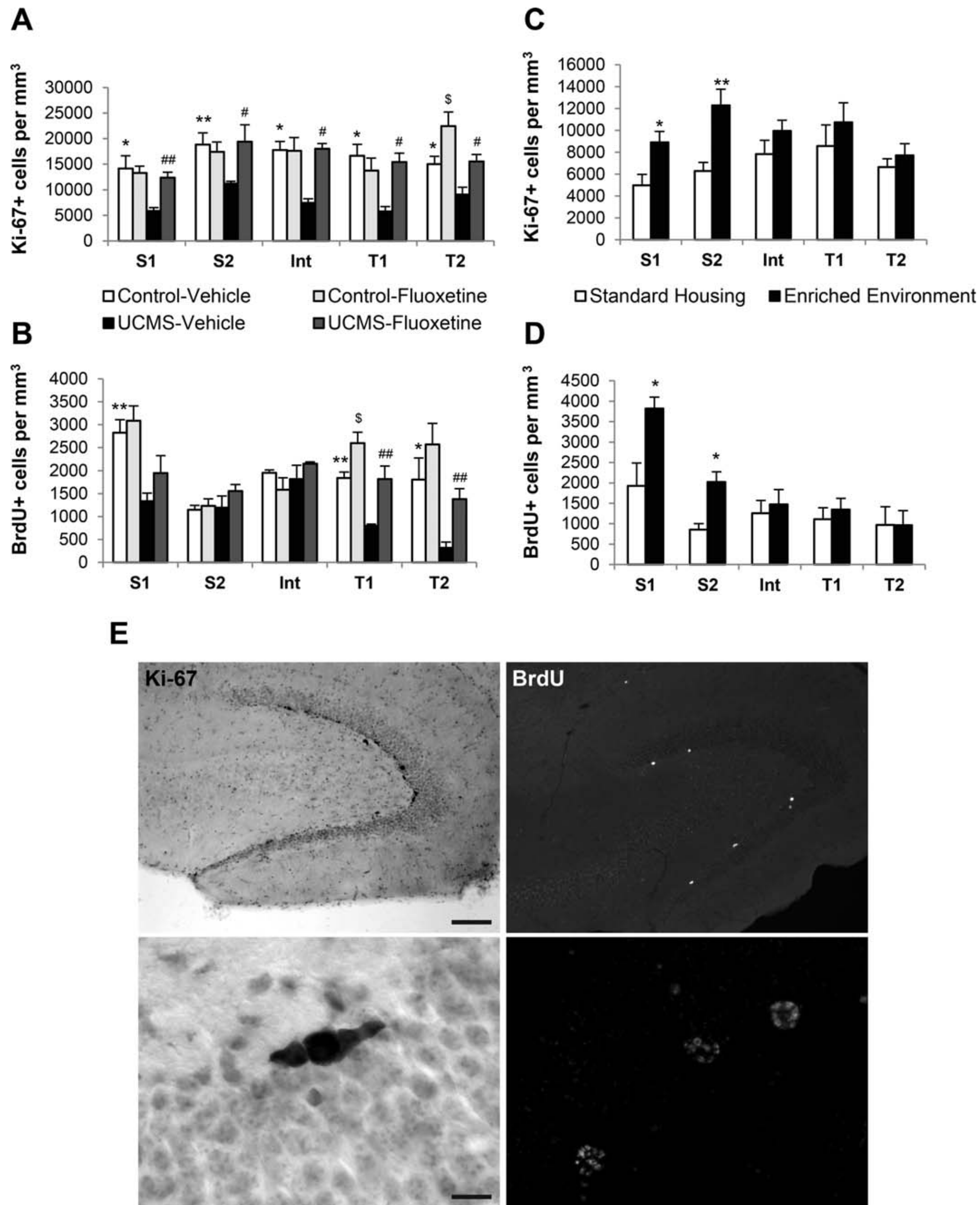


FIGURE 2. Effects of UCMS, fluoxetine treatment and EE on cell proliferation and cell survival in the dentate gyrus along the septo-temporal axis of the hippocampus. A: UCMS decreased the density of Ki-67+ cells all along the septo-temporal axis. Fluoxetine treatment reversed all the UCMS-induced effects but in control mice increased the density of ki-67 cells specifically in the T2 division. B: The density of BrdU+ 4-week-old newborn cells was decreased by UCMS in both septal (S1) and temporal (T1 and T2) divisions. These effects were reversed by fluoxetine treatment only in the temporal divisions whereas in control mice fluoxetine treatment increased the density of BrdU+ cells only in the T1 division. C: EE increased the density of Ki-67+ cells specifically in both septal divisions (S1 and S2). D: EE increased the density of BrdU+ cells specifically in the septal divisions. Data represent mean ±SEM, $n = 6$ per group. Kruskall Wallis followed by Mann Whitney U test: $^{**}P < .01$; $^{*}P < 0.05$ for Control-Vehicle versus UCMS-Vehicle groups or Standard Housing versus Enriched Environment; $\#P < 0.05$ for UCMS-Vehicle versus UCMS-Fluoxetine groups; \$: $P < 0.05$ for Control-Vehicle versus Control-Fluoxetine groups. E: Representative photographs of the Ki-67 and BrdU immunolabeling in a Standard Housing mouse. Scale bar: 100 μm (upper lane) and 15 μm (lower lane).

2008; Surget et al., 2011; Nollet et al., 2012). Additionally, serum fluoxetine levels following chronic treatment with both these doses (Dulawa et al., 2004) were found to be toward the bottom and the high end, respectively, of plasma concentrations found in patients taking 20–80 mg/day fluoxetine (Koran et al., 1996), which is the clinical range used to treat major depressive disorders. This UCMS paradigm has been shown to induce reliable impairments of hippocampal

neurogenesis in BALB/C mice, which are prevented by fluoxetine treatment (Surget et al., 2008; Nollet et al., 2012; Tanti et al., 2012).

Antidepressant treatments in previous studies from our group were performed 2 weeks after the start of the stress procedure in order to mimic a situation where subjects are already submitted to a pathological condition before starting the therapy. However, having a delay between stress and treatment could have blurred the analysis of neurogenesis as treatment and stress could have affected distinct cell populations. In this case, cells that could benefit from treatment would already be 2 weeks old, which might be too late for fluoxetine to reverse the impairments induced by UCMS prior to treatment. To benefit from clearer measures, we therefore chose to start treatment with the stress procedure and shortened the duration of the UCMS protocol in order to avoid this bias.

## Environmental Enrichment

A second cohort of animals was used for the enrichment experiment (Fig. 1A). Mice were divided into 2 groups: Enriched Environment and Standard Housing ($n = 8$ per group). Enrichment was conducted as previously described (Tanti et al., 2012) for a period of four weeks. Enriched mice were group-housed (5 per cage) in larger cages than controls ($53 \times 38 \times 26$ cm$^3$) containing objects such as plastic tubes in which mice could climb and use to navigate through the cage, a running wheel, rodent dwellings and nesting material. Various novel objects of different shape and size were added two times a week in order to stimulate interest toward novelty. Each time, two copies of the same object were placed each in different areas of the cage to make sure that all animals were given the opportunity to explore them and to decrease conflicting social interactions.

Mice had access to food and water ad libidum. This paradigm has been shown to reliably stimulate neurogenesis in BALB/C mice (Tanti et al., 2012).

## BrdU Labeling

In order to label and assess the survival of 4-week-old newborn cells, all animals were given 4 daily i.p injections spaced 2 h apart with the thymidine analogue 5-bromo-2'-deoxyuridine (BrdU; $4 \times 75$ mg/kg prepared in saline; Sigma-Aldrich) for 2 days prior to the start of the UCMS procedure.

## Tissue Processing and Immunohistochemistry

After deep anesthesia (Sodium pentobarbital, 40 mg/kg), mice were transcardially perfused with heparinized saline (0.9% sodium chloride, 1000 UI heparin) for 2 min followed by 4% paraformaldehyde (PFA)/0.1 M phosphate-buffered saline (PBS, pH 7.4) for 5 min. Brains were collected and post-fixed 2 h in 4%PFA/0.1 M PBS at 4°C, and then cryoprotected in 20% sucrose/0.1 PBS at 4°C. To optimize the septo-temporal dissociation, serial horizontal sections (40 μm thick) were cut with a cryostat (Leica CM 3050S) and every fourth section was collected and stored in PBS (0.1 M, pH 7.4) until free-floating processing.

Different markers were then used to identify and quantify newborn cells (Fig. 1B). BrdU was used to label 4-week-old cells and assess changes in cell survival following UCMS, fluoxetine treatment and enrichment. Ki-67 was used to label proliferative cells. Triple labeling for DCX, Prox1 and CR (Fig. 3G) was then performed to quantify neuronal progenitors (DCX+/Prox1+ cells) and post-mitotic neurons (DCX+/Prox1+/CR+ and DCX-/Prox1+/CR+ cells). Since CR is expressed in immature neurons but also in inter-neurons and mossy cells Prox1 was mainly used as a control to specify the neuronal phenotype of CR+ cells.

Immunohistochemistry for the proliferation marker Ki-67 was visualized with DAB. Sections were treated in 3% H2O2/50% ethanol for 20 min, rinsed in PBS (0.1 M, pH 7.4) and incubated with a rabbit anti-Ki-67polyclonal antibody (1:1000, Abcam) for 40 h at room temperature in blocking solution (0.1M PBS/0.2% Triton/3% horse serum). Sections were then rinsed and incubated 2 h with a donkey anti-rabbit biotinylated antibody (1:500, Jackson ImmunoResearch) in blocking solution followed by amplification with an avidin–biotin complex (Elite ABC kit, Vector Laboratories) and visualized with DAB (Sigma–Aldrich). After washing with PBS, sections were mounted on gelatin-coated slides, dried, dehydrated, and coverslipped.

For fluorescent BrdU labeling sections were first treated with 2 N HCL for 45 min at room temperature, rinsed in PBS, and incubated overnight with a rat anti-BrdU antibody (1:200, Santa Cruz) in blocking solution at room temperature. After washing in PBS, sections were incubated with a donkey anti-rat fluorochrome-conjugated Alexa-488 antibody (1:500, Invitrogen) for 2 h at room temperature, rinsed, and mounted on slides with Vectashield mounting medium (Vector Laboratories).

For DCX, Prox1 and Calretinin triple labeling sections were incubated for 40 h at room temperature with the following antibodies: goat anti-DCX (1:500, Santa Cruz), rabbit anti-Prox1 (1:1500, Abcam) and mouse anti-Calretinin (1:500, Swant) in blocking solution. After rinsing with PBS sections were incubated for 2 h at room temperature with the following secondary antibodies: Alexa-488 donkey anti-goat, Alexa-555 donkey anti-rabbit and Alexa-647 donkey anti-mouse (1:500, Invitrogen) in blocking solution. Sections were then rinsed and mounted onto slides under Vectashield mounting medium (Vector Laboratories). Different negative controls were performed by omitting primary or secondary antibodies.

## Topographical Division of the Hippocampus Along the Septo-Temporal Axis and Cell Counting

To quantify regional changes in neurogenesis following UCMS, fluoxetine treatment or environmental enrichment horizontal sections were assigned to five subdivisions (Fig. 1C)

along the dorsoventral axis of the brain: Septal 1 (∼−2.04 mm below bregma, anterior half of the dentate gyrus), Septal 2 (∼−2.04 mm below bregma, posterior half of the dentate gyrus), Intermediate (∼−2.36 mm below bregma), Temporal 1 (∼−3.44 mm below bregma) and Temporal 2 (∼−4.28 mm below bregma). Because of the curvature of the hippocampus which extends rostro-caudally and also dorso-ventrally, this allowed us to segregate septal and temporal divisions of the dentate gyrus without the drawbacks of extracting the whole hippocampus from the brain and maybe more precisely than coronal sections. Indeed while anterior coronal sections accurately include the septal hippocampus, posterior sections include both temporal (ventrally located) but also what can be considered dorsal to intermediate divisions of the hippocampus. While horizontal sections have the same drawback in the dorsal most sections where the hippocampus extends horizontally (Fig. 1C, Septal 1 and 2, ∼−2.04 mm below bregma), given the shape of the dentate gyrus in such sections it is however easier to segregate its anterior and posterior parts.

Cell counting and density measurements were performed with an epifluorescence microscope with ApoTome (Imager. Z2, Zeiss) and AxioVision software (Zeiss) under a x40 lens. For each regional subdivision, two successive sections per animal were used and both hippocampi per section were included in the analysis (4 hippocampi per region). For each section, *z*-stacks of the dentate gyrus with a total thickness of 20 μm per stack were acquired with a 1-μm interval between each image (21 *z*-images for each stack). Every positive cell within this 20 μm thickness of the granule cell layer was counted and the density of labeled cells for each section was calculated by dividing the number of positive cells by the respective volume of the dentate gyrus (surface x 20 μm). Values of representative dentate gyrus for each animal were then averaged to yield the density of labeled cells for each subdivision. Colocalization of DCX, Prox1 and CR were verified for each cell in *z*-stacks using planes in which the soma of the cell was included.

### Statistical Analysis

Given that the assumptions for parametric analyses were not ensured (normality and homoscedasticity, respectively assessed with Shapiro-Wilk and Levene's tests) nonparametric statistical tests were performed. Between groups effects were assessed by the Kruskal-Wallis one-way ANOVA by ranks followed by the Mann-Whitney U test for two-by-two comparisons. For Standard Housing versus Enriched Environment comparisons, the Mann-Whitney U test was directly applied.

Differences in neurogenesis and maturation between the topographical subdivisions of the hippocampus for each group were assessed by Friedman's repeated measures ANOVA by ranks followed by Wilcoxon's signed rank test for two-by-two comparisons. Significance threshold was set at $P < 0.05$. All data are expressed as mean ± standard error of the mean (SEM).

## RESULTS

### Chronic Stress, Fluoxetine Treatment, and Environmental Enrichment Differentially Regulate Cell Proliferation and Survival Along the Septo-Temporal Axis of the Hippocampus

To assess the regional changes in cell proliferation induced by UCMS and fluoxetine, the density of Ki-67 positive cells was quantified along the septo-temporal axis of the hippocampus (Fig. 2A). Kruskal Wallis H-test revealed a significant effect of group for all subdivisions of the hippocampus (S1: $H(3, 24) = 9.44$, $P < 0.05$; S2: $H(3, 24) = 7.98$, $P < 0.05$; Intermediate: $H(3, 24) = 9.08$, $P < 0.05$; T1: $H(3, 24) = 8.12$, $P < 0.05$; T2: $H(3, 24) = 10.01$, $P < 0.05$; Fig. 2A). Post-hoc comparisons indicated that exposure to UCMS significantly reduced the density of dividing cells all along the septo-temporal axis (S1: $P < 0.05$; S2: $P < 0.01$; Intermediate: $P < 0.05$; T1 and T2: $P < 0.05$; UCMS-Vehicle versus Control-Vehicle, Fig. 2A). These effects were all reversed by fluoxetine in UCMS mice (S1: $P < 0.01$; S2: $P < 0.05$; Intermediate: $P < 0.05$; T1: $P < 0.05$; T2: $P < 0.05$; UCMS-Fluoxetine versus UCMS-Vehicle, Fig. 2A). However, chronic fluoxetine treatment in control mice stimulated proliferation only in the most temporal part of the hippocampus (T2: $P < 0.05$; Control-Fluoxetine versus Control-Vehicle, Fig. 2A).

Regional changes in cell survival were assessed by quantifying the density of BrdU positive cells (4-week-old newborn cells; Fig. 2B). Differences between groups were found in the most septal and both temporal subdivisions (S1: $H(3, 24)=11.39$, $P < 0.01$; S2: $H(3, 24)=3.569565$, $P > 0.05$; Intermediate: $H(3, 24)=3.05$, $P > 0.05$; T1: $H(3, 24)=14.04$, $P < 0.01$; T2: $H(3, 24)=11.47$, $P < 0.01$; Fig. 2B). UCMS significantly reduced the density of BrdU positive cells in these subdivisions (S1: $P < 0.01$; T1: $P < 0.01$; T2: $P < 0.05$; UCMS-Vehicle versus Control-Vehicle, Fig. 2B), indicating that the effects of UCMS on cell proliferation and cell survival are both found in septal and temporal parts of the hippocampus. However, concomitant treatment with fluoxetine in UCMS mice only reversed this decrease in the temporal subdivisions of the hippocampus (T1: $P < 0.01$; T2: $P < 0.01$; UCMS-Fluoxetine versus UCMS-Vehicle, Fig. 2B). In control mice, fluoxetine treatment stimulated cell survival only in the first temporal division (T1: $P < 0.05$; Control-Fluoxetine versus Control-Vehicle, Fig. 2B).

Interestingly, housing in the enriched environment stimulated both cell proliferation (S1: $P < 0.05$; S2: $P < 0.01$; Enriched Environment versus Standard Housing, Fig. 2C) and cell survival (S1: $P < 0.05$; S2: $P < 0.05$; Enriched Environment versus Standard Housing, Fig. 2D) only in the septal divisions of the hippocampus.

### Region and Stage-Specific Effects of Chronic Stress, Fluoxetine Treatment, and Enrichment on Neurogenesis

To further specify these effects and investigate whether chronic stress, antidepressant treatment and environmental

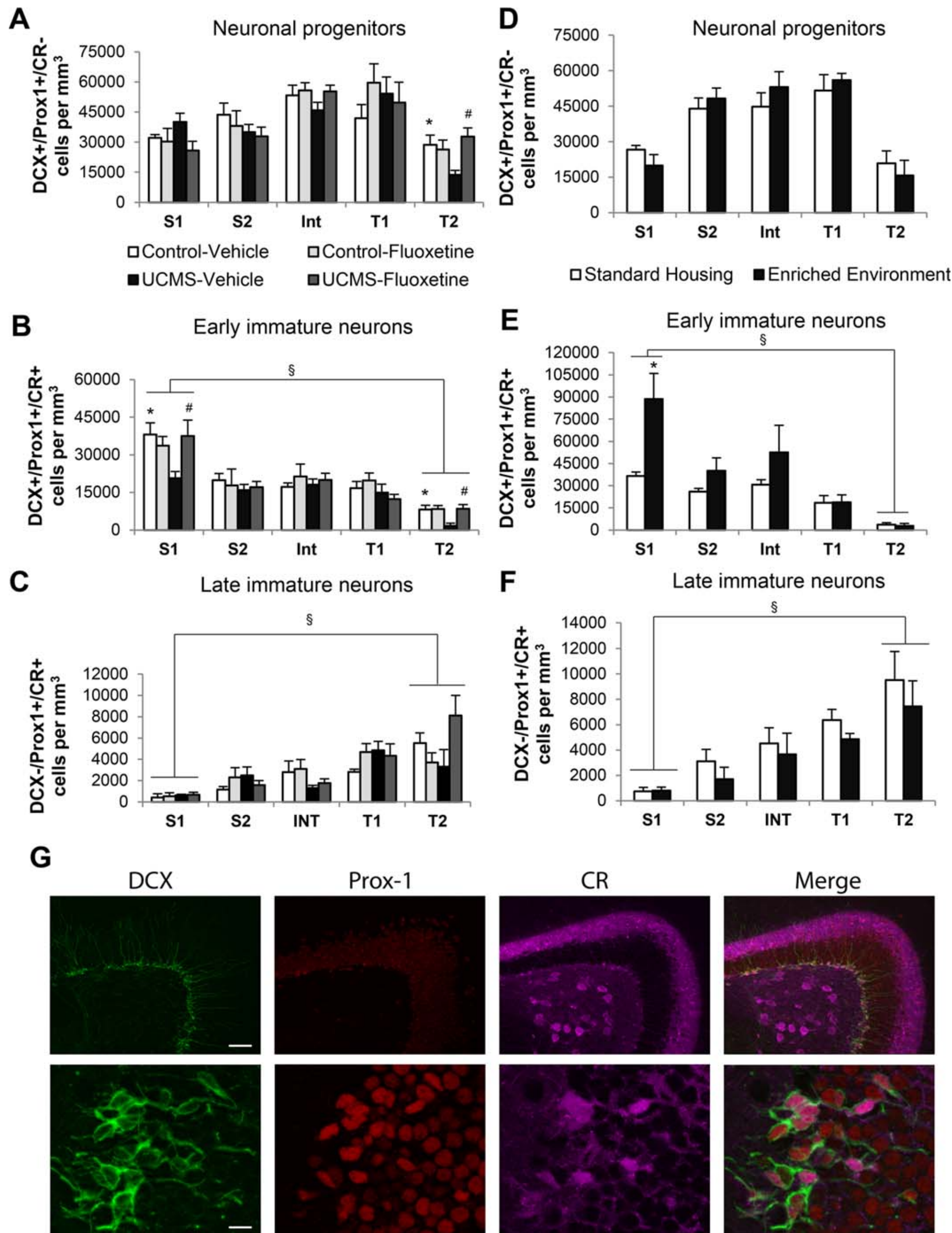


FIGURE 3. Effects of UCMS, fluoxetine treatment and EE on the number of neuronal progenitors and post-mitotic immature neurons along the septo-temporal axis of the hippocampus. A: The density of DCX+/Prox-1+/CR- neuronal progenitors was decreased by UCMS specifically in the T2 division. This effect was reversed by fluoxetine but no effect of treatment was found in control animals. B: UCMS decreased the density of DCX+/Prox-1+/CR+ early immature neurons in both S1 and T2 divisions. This effect was reversed by fluoxetine but no effect of treatment was found in control animals. C: No effect of UCMS and fluoxetine was found on the density of DCX-/Prox-1+/CR+ late immature neurons all along the septo-temporal axis. D: No effect of EE on the density of neuronal progenitors was found along the septo-temporal axis of the hippocampus. E: EE increased the density of early immature neurons specifically in the S1 division. F: No effect of enrichment was found on the density of late immature neurons all along the septo-temporal axis. B, C, E, F: A higher density of early and late immature neurons was found in the S1 division compared to the T2 division for all groups. Data represent mean ±SEM, $n = 4$ per group. Kruskall Wallis followed by Mann Whitney U test: $*P < 0.05$ for Control-Vehicle versus UCMS-Vehicle groups or Standard Housing versus Enriched Environment; $\#P < 0.05$ for UCMS-Vehicle versus UCMS-Fluoxetine groups; Friedman followed by Wilcoxon's two-by-two comparisons: §: $P < 0.05$ for S1 versus S2 for all groups. G: Representative photographs of the DCX/Prox-1/CR triple labeling in a Standard Housing mouse. Scale bar: 50 μm (upper lane) and 15 μm (lower lane). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

enrichment affected different populations of newborn cells and whether these effects were similar along the septo-temporal axis of the hippocampus, triple labeling for DCX, Prox1, and CR was performed (Fig. 3). Three distinctive subpopulations according to their stage of maturation were quantified: type 2b/3 progenitor cells (DCX+/Prox1+/CR−), early immature neurons that reached a post-mitotic stage (DCX+/Prox1+/CR+) and late post-mitotic newborn neurons expressing CR but not DCX (DCX-/Prox1+/CR+).

Regarding the effects of UCMS and fluoxetine on progenitor cells (DCX+/Prox1+/CR−), Kruskal Wallis H-test revealed a significant effect of group only in the most temporal subdivision of the hippocampus (S1: $H(3, 16) = 4.92$, $P > 0.05$; S2: $H(3, 16) = 1.7$, $P > 0.05$; Intermediate: $H(3, 16) = 3.79$, $P > 0.05$; T1: $H(3, 16) = 2.56$, $P > 0.05$; T2: $H(3, 16) = 8.933824$, $P < 0.05$; Fig. 3A). Post-hoc comparisons indicated that exposure to UCMS significantly reduced the density of progenitor cells in this subdivision (T2: $P < 0.05$; UCMS-Vehicle versus Control-Vehicle, Fig. 3A), an effect that was prevented by fluoxetine treatment (T2: $P < 0.05$; UCMS-Fluoxetine versus UCMS-Vehicle, Fig. 3A). However, no significant effect of treatment was found in control animals (T2: $P > 0.05$; Control-Fluoxetine versus Control-Vehicle, Fig. 3A).

Whereas changes in progenitor cells were found only in the most temporal division of the hippocampus, significant effect of group on the density of early immature neurons (DCX+/Prox1+/CR+) was found both in the most septal and the most temporal subdivisions (S1: $H(3,16)= 8.052$, $P < 0.05$; S2: $H(3, 16) = 2.05$, $P > 0.05$; Intermediate: $H(3, 16) = 0.64$, $P > 0.05$; T1: $H(3, 16) = 3.86$, $P > 0.05$; T2: $H(3,16)=7{,}82$, $P < 0.05$; Fig. 3B). UCMS exposure decreased the density of early immature neurons in both these divisions (S1: $P < 0.05$; T2: $P < 0.05$; UCMS-Vehicle versus Control-Vehicle, Fig. 3B). The effects of UCMS were reversed by fluoxetine treatment (S1: $P < 0.05$; T2: $P < 0.05$; UCMS-Fluoxetine versus UCMS-Vehicle, Fig. 3B) but no effect of treatment was found in control mice (S1: $P > 0.05$; T2: $P > 0.05$; Control-Fluoxetine versus Control-Vehicle, Fig. 3B).

Last, no difference between groups was found on the density of late immature neurons (DCX-/Prox1+/CR+) (S1: $H(3,16) = 1.19$, $P > 0.05$; S2: $H(3, 16) = 2.91$, $P > 0.05$; Intermediate: $H(3, 16) = 3.07$, $P > 0.05$; T1: $H(3, 16) = 5.38$, $P > 0.05$; T2: $H(3, 16) = 4.76$, $P > 0.05$; Fig. 3C).

Regarding the effects of enrichment, no change in the density of progenitor cells was found (Mann Whitney U test, $P > 0.05$ for all subdivisions; Enriched Environment versus Standard Housing, Fig. 3D). However, enrichment stimulated the density of early immature neurons specifically in the most septal division of the hippocampus (S1: $P < 0.05$; Enriched Environment versus Standard Housing, Fig. 3E), without affecting the density of late immature neurons ($P > 0.05$ for all subdivisions; Enriched Environment versus Standard Housing, Fig. 3F).

## Gradients of Neurogenesis and Maturation Along the Septo-Temporal Axis of the Hippocampus

Gradients of the different cell populations along the septo-temporal axis of the hippocampus were analyzed with Friedman's repeated measures ANOVA by ranks followed by Wilcoxon's signed-rank test for two-by-two comparisons (when a significant effect of subdivision was found. For every group, no significant effect of septo-temporal location was found on the density of Ki-67+ cells (Friedman's ANOVA: Control-Vehicle: $\chi^2(4, 6)= 1.4$, $P > 0.05$; Control-Fluoxetine: $\chi^2(4, 6) = 4$, $P > 0.05$; UCMS-Vehicle: $\chi^2(4, 6) = 6.4$, $P > 0.05$; UCMS-Fluoxetine: $\chi^2(4, 6)= 5.6$, $P > 0.05$, Fig. 2A; Standard Housing: $\chi^2(4, 6) = 7.46$, $P > 0.05$; Enriched Environment: $\chi^2(4, 6) = 6.4$, $P > 0.05$, Fig. 2C), BrdU+ cells (Control-Vehicle: $\chi^2(4, 5)= 5.2$, $P > 0.05$; Control-Fluoxetine: $\chi^2(4, 6) = 8.13$, $P > 0.05$; UCMS-Vehicle: $\chi^2(4, 6) = 8.46$, $P > 0.05$; UCMS-Fluoxetine: $\chi^2(4, 6)= 2.93$, $P> 0.05$, Fig. 2B; Standard Housing: $\chi^2(4, 6) = 3.6$, $P > 0.05$; Enriched Environment: $\chi^2(4, 6) = 8.6$, $P > 0.05$, Fig. 2D) and progenitor cells (Control-Vehicle: $\chi^2(4, 4)= 7$, $P > 0.05$; Control-Fluoxetine: $\chi^2(4, 4) = 8.2$, $P > 0.05$; UCMS-Vehicle: $\chi^2(4, 4) = 8.6$, $P > 0.05$; UCMS-Fluoxetine: $\chi^2(4, 4)= 9$, $P > 0.05$, Fig. 3A; Standard Housing: $\chi^2(4, 4) = 9.43$, $P > 0.05$; Enriched Environment: $\chi^2(4, 4) = 8.87$, $P > 0.05$, Fig. 3D).

However, differences along the subdivisions of the hippocampus were found for the density of early immature neurons in all groups (Control-Vehicle: $\chi^2(4, 4)= 13.4$, $P < 0.01$; Control-Fluoxetine: $\chi^2(4, 4) = 11.8$, $P < 0.05$; UCMS-Vehicle: $\chi^2(4, 4) = 10$, $P < 0.05$; UCMS-Fluoxetine: $\chi^2(4, 4)= 12.6$, $P < 0.05$, Fig. 3B; Standard Housing: $\chi^2(4, 4) = 12.4$, $P < 0.05$; Enriched Environment: $\chi^2(4, 4) = 11.47$, $P < 0.05$, Fig. 3E), with a lower density of DCX+/Prox1+/CR+ cells in the last temporal division compared with the most septal one (Wilcoxon's signed-rank tests: S1 versus T2: $P < 0.05$ for all groups, Figs. 3B,E).

Inversely, the density of late immature neurons was also significantly affected by septo-temporal location (Control-Vehicle: $\chi^2(4, 4)= 12$, $P < 0.05$; Control-Fluoxetine: $\chi^2(4, 4) = 13.2$, $P < 0.05$; UCMS-Vehicle: $\chi^2(4, 4) = 9.6$, $P < 0.05$; UCMS-Fluoxetine: $\chi^2(4, 4)= 13.4$, $P<0.01$, Fig. 3C; Standard Housing: $\chi^2(4, 4) = 12$, $P < 0.05$; Enriched Environment: $\chi^2(4, 4) = 11.8$, $P<0.05$, Fig. 3F), but with a higher density of DCX-/Prox1+/CR+ cells in the last temporal division compared with the most septal one (S1 versus T2: $P<0.05$ for all groups, Figs. 3C,F).

This is consistent with a shift toward an increase in the proportion of progenitor cells (Wilcoxon signed-rank tests: S1 versus T2: $P < 0.05$ for all groups except UCMS-Vehicle, Figs. 4A,B) and a decreased proportion of DCX+ cells that reached a post-mitotic stage and coexpressed CR in the temporal hippocampus (S1 versus T2: $P < 0.05$ for all groups, Figs. 4A,B).

Regarding the effects of UCMS and fluoxetine, the proportions of progenitor cells (S1: $H(3, 16) = 7.83$, $P < 0.05$; T2: $H(3, 16) = 0.88$, $P > 0.05$; Fig. 4A) and early immature

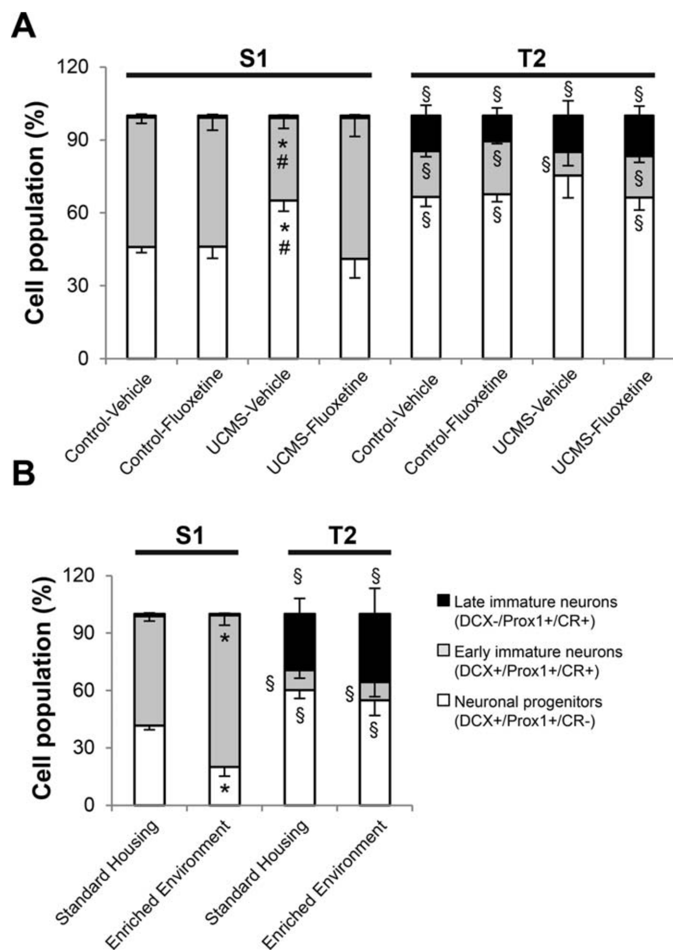


**FIGURE 4. Effects of UCMS, fluoxetine treatment and EE on the proportions of progenitor cells (DCX+/Prox1+/CR-), early post-mitotic neurons (DCX+/Prox1+/CR+) and late immature neurons (DCX-/Prox1/CR+) along the septo-temporal axis of the hippocampus. A: UCMS decreased the proportion of DCX+/Prox1+ that reached a more mature stage and coexpressed CR and increased the proportion of progenitor cells (DCX+/Prox1+/CR-) cells specifically in the S1 division. This effect was reversed by fluoxetine but no effect of treatment was found in control animals. B: EE increased the proportion of DCX+/Prox-1+ cells coexpressing the more mature marker CR and decreased the proportion of progenitor cells specifically in the S1 division. A, B: There was a lower proportion of early immature neurons (DCX+/Prox1+/CR+) and a higher proportion of late immature neurons (DCX-/Prox1+/CR+) in the T2 division of the hippocampus for all groups compared with S1. The proportion of progenitors (DCX+/Prox1+/CR-) was higher in the T2 division compared with S1 for all groups but not in UCMS-Control mice. Data represent mean ±SEM, *n* = 4 per group. Kruskall Wallis followed by Mann Whitney U test: *: *P* < 0.05 for Control-Vehicle versus UCMS-Vehicle groups or Standard Housing versus Enriched Environment; #: *P* < 0.05 for UCMS-Vehicle versus UCMS-Fluoxetine groups; Friedman followed by Wilcoxon's two-by-two comparisons: §: *P* < 0.05 for S1 versus S2 for all groups.**

neurons (S1: $H(3, 16) = 7.87$, $P < 0.05$; T2: $H(3, 16) = 5.1$, $P > 0.05$; Fig. 4A) were significantly different between groups only in the septal hippocampus. UCMS exposure increased the proportion of progenitor cells in the septal hippocampus (S1: $P < 0.05$; UCMS-Vehicle versus Control-Vehicle, Fig. 4A) and decreased the proportion of early post-mitotic neurons (S1: $p<0.05$; UCMS-Vehicle versus Control-Vehicle, Fig. 5A). Both these effects were reversed by fluoxetine treatment (S1: $P < 0.05$; UCMS-Fluoxetine versus UCMS-Vehicle, Fig. 4A) but no effect of treatment was found in controls (S1: $P > 0.05$; Control-Fluoxetine versus Control-Vehicle, Fig. 4A).

Environmental enrichment had opposite effects than those of UCMS and increased the proportion of DCX+ cells that reached a more mature stage and coexpressed CR only in the septal hippocampus (S1: $P < 0.05$; T2: $P > 0.05$; Enriched Environment versus Standard Housing, Fig. 4B) whereas it decreased the proportion of progenitor cells (S1: $P < 0.05$; T2: $P > 0.05$; Enriched Environment versus Standard Housing, Fig. 4B).

## DISCUSSION

This study was conducted to investigate whether different environmental manipulations known to modulate cognitive and emotional hippocampal functions would induce regional and stage-specific changes in neurogenesis along the septo-temporal axis of the hippocampus.

Results are summarized in Table 1. We found that: (1) enrichment stimulated cell proliferation, increased the survival of newborn cells and increased the density and proportion of DCX+ cells that reached a post-mitotic stage and co-expressed CR in the septal hippocampus. (2) Inversely, fluoxetine treatment in control mice stimulated proliferation and cell survival only in the temporal hippocampus without affecting the number of progenitors or immature neurons. (3) UCMS decreased cell proliferation, the number of early post-mitotic neurons, and survival of newborn cells all along the septo-temporal axis, while reducing progenitors only in the temporal pole. Those UCMS-induced effects were almost all reversed by fluoxetine treatment. (4) Last, there was a decrease in the number and proportion of early immature neurons in the temporal hippocampus compared to septal divisions, while the population of late immature neurons expressing CR but not DCX gradually increased in the temporal hippocampus.

Region-specific effects of environmental factors could be linked to gradients of neurogenesis and maturation as well as distinct functional properties of newborn neurons in the septal and temporal hippocampus. In our study, despite no difference between septal versus temporal subdivisions in the number of proliferative cells, neural progenitors or four week-old newborn cells, there was a lower number of immature neurons in the temporal pole of the hippocampus and a decrease in the proportion of progenitors reaching a more mature stage and expressing the post-mitotic marker CR. This suggests a slower rate of maturation or cell cycle exit of newborn neurons in the temporal hippocampus. Consistent with our results, gradients

*TABLE 1.*

***Summary of the Effects Induced by EE, 4 Weeks Fluoxetine Treatment (20 mg/kg, ip) and UCMS on Different Steps of Neurogenesis***

| | Ki67 (cell proliferation) | | BrdU (cell survival) | | DCX+/Prox1+/CR-(neuronal progenitors) | | DCX+/Prox1+/CR+ (early post-mitotic immature neurons) | | DCX-/Prox1+/CR+ (late post-mitotic immature neurons) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Septal = Temporal | | Septal = Temporal | | Septal = Temporal | | Septal > Temporal | | Septal < Temporal | |
| EE | ↗↗ | 0 | ↗↗ | 0 | 0 | 0 | ↗ | 0 | 0 | 0 |
| Fluoxetine | 0 | ↗ | 0 | ↗ | 0 | 0 | 0 | 0 | 0 | 0 |
| UCMS - Fluoxetine | ↘↘-R | ↘↘-R | ↘ | ↘↘-R | 0 | ↘-R | ↘-R | ↘-R | 0 | 0 |

Arrows indicate a significant decrease (↘) or increase (↗) in the density of the respective population assessed. The number of arrows corresponds to the number of septal or temporal divisions in which changes were observed. R indicates that the UCMS-induced effects were reversed by fluoxetine treatment. (=), (>) or (<) indicate respectively similar densities in the septal and temporal divisions, higher or lower densities in the septal hippocampus.

of neurogenesis (Tashiro et al., 2007; Snyder et al., 2009; Jinno, 2011) as well as gradients in maturation of newborn neurons along the septo-temporal axis of the hippocampus (Piatti et al., 2011; Snyder et al., 2012) have already been described. By using morphological and electrophysiological characterizations in combination with endogenous markers, Piatti et al. (2011) showed that new neurons in the temporal hippocampus mature more slowly than in the septal hippocampus and that this could be linked to differences in the intrinsic excitability of newborn neurons and local changes in network activity. Given the higher excitability of newborn neurons compared to mature granule cells (Mongiat and Schinder, 2012), different rates of maturation between septal and temporal poles of the hippocampus may thus give rise to different cohorts of newborn neurons which could differentially respond to environmental situations and contribute to hippocampal functions. Supporting the view that environmental factors could differentially affect the developmental stages of newborn neurons along the septo-temporal axis, we found that in the septal hippocampus chronic stress decreased the density of early post-mitotic immature neurons without affecting the pool of neuronal progenitors. In the temporal hippocampus however, chronic stress exposure decreased both progenitors and post-mitotic immature neurons. In addition, the decreased proportion of DCX+ cells co-expressing CR following UCMS in the septal hippocampus may indicate that chronic stress decreases the rate of maturation of progenitors into immature neurons specifically in that region but affects anterior stages of neuronal development in the temporal hippocampus. Alternatively, changes in the number of immature neurons in the septal hippocampus may also be linked to a stage-specific decrease in cell survival rather than changes in the maturation of newborn neurons, even if our experiment does not allow to identify the cell populations in which survival would be more affected by stress and enrichment, reflecting the changes in BrdU+ cells we observed. Additional indices of maturation, such as assessment of the morphological properties of DCX+ cells dendritic arborization could have helped dissociating these effects. It is nonetheless the first study to our knowledge to report stage-specific effects of chronic stress on neurogenesis along the septo-temporal axis of the hippocampus.

Similarly, we found that enrichment preferentially increased the number of immature neurons without affecting the number of neural progenitors and consequently increased the proportion of early post-mitotic neurons specifically in the dentate gyrus of the septal hippocampus. The combination of CR and DCX markers has previously been used to assess changes in maturation and cell cycle exit (Brandt et al., 2010) following voluntary exercise. In particular physical exercise was shown to increase the proportion of DCX+ cells reaching a more mature stage and expressing CR (Brandt et al., 2010). Our results are in accordance with that study but indicate that those effects are restricted to the septal part of the hippocampus. Interestingly, UCMS had opposite effects than enrichment and induced a shift toward a decreased proportion of early post-mitotic neurons, also in the septal hippocampus.

The fact that some studies have reported drops in the expression of CR in the temporal hippocampus of control mice (Liu et al., 1996; Jinno, 2011), as well as changes in its expression in some neurodegenerative pathologies or various conditions (Baglietto-Vargas et al., 2010; Steullet et al., 2010; Volz et al., 2011; Xu et al., 2011; Maskey et al., 2012) may suggest that CR might not be optimal to assess regional changes in neurogenesis or maturation. Gradients of coexpression of DCX and CR along the septo-temporal hippocampus in control animals could indeed reflect this drop of CR expression rather than differences in maturation. However, despite these reports, we found an important number of CR+ cells in the temporal hippocampus and their neuronal phenotype was specified by coexpression of the granular cell marker Prox-1.

Notably, whereas in the septal hippocampus almost every CR+ cell coexpressed DCX, in the temporal hippocampus an increased proportion of CR+ cells did not express DCX, maybe suggesting that newborn neurons in the temporal hippocampus express CR for a longer period of time, thus confirming a differential rate of maturation along the septo-temporal axis. Alternatively, newborn neurons in the temporal hippocampus might actually mature faster out of the DCX-expressing time window. In our experiment however, neither stress nor enrichment and antidepressant treatment affected the number CR+ cells not expressing DCX. This stage specificity and lack of effect on late immature neurons may reflect a higher susceptibility to environmental changes and network activity during earlier stages of maturation. This could possibly be linked to transient changes in the expression of glucocorticoid receptors (Garcia et al., 2004) or CREB phosphorylation and signaling (Jagasia et al., 2009) occurring at specific stages in the development of newborn neurons. In view of these results, further characterization of the electrophysiological and molecular specificities of CR-expressing cells may allow a better understanding as to how environmental and pharmacological manipulations may shape adult neurogenesis.

Nonetheless, the reduced or increased number of progenitor cells and early immature neurons we observed following UCMS or enrichment would be expected to translate into changes in number of late immature neurons. Similarly, our results show that changes in the number of proliferative and 4-week-old newborn cells following UCMS or EE exposure do not necessarily correlate with changes in the number of mitotic DCX+ neural progenitors. Additionally, while fluoxetine reversed the UCMS-induced effects and stimulated cell proliferation and survival in the temporal hippocampus of control mice, it did not affect the number of progenitors or immature neurons in neither septal nor temporal divisions. Consistent with these results, Wang et al. (2008) previously showed that chronic fluoxetine treatment increased the number of proliferative cells and the survival of newborn cells in the dentate gyrus without affecting the total number of DCX+ cells. Rather, fluoxetine increased the proportion of new neurons displaying a mature phenotype and shortened the time window of DCX expression; thus it increased the rate of maturation of newborn neurons. Other studies also showed that an increased number of proliferative cells and new mature neurons following antidepressant treatment could be associated with decreased numbers of immature neurons (Banasr et al., 2006; Klempin et al., 2010). Cumulative and confounding effects of fluoxetine, EE and UCMS on distinct steps in the development of newborn neurons may therefore have blurred some of our results. For example, by respectively decreasing or enhancing the proliferation of progenitors but also their rate of maturation into post-mitotic stage (and therefore their timing of CR expression), UCMS and enrichment would not modify the net population of progenitors as both the cells that enter and those that exit the cell cycle would be affected. Similarly, fluoxetine has previously been shown to enhance neurogenesis by specifically targeting and enhancing the proliferation of Nestin+ early progenitors (Encinas et al., 2006), which were not quantified in our experiment. Combined with a possible reduced length of maturation of their progenies, this might explain the absence of effects we observe on progenitors and immature neurons following chronic fluoxetine treatment, despite an increased number of 4-week-old BrdU+ cells.

While additional stage-specific markers may therefore be required to fully understand the precise changes in distinct newborn cell populations following UCMS, fluoxetine treatment or enrichment, it is noteworthy that these effects occurred differentially along the septo-temporal axis of the hippocampus. In particular, UCMS affected progenitor cells only in the most temporal division of the hippocampus but reduced the density of early post-mitotic neurons in both septal and temporal areas. This highlights that, depending on which cell population that is addressed, stress can impair neurogenesis all long the septo-temporal axis of the hippocampus. Maybe due to basal differences in the rates of maturation of newborn neurons between septal and temporal divisions, the dynamic changes observed following UCMS and antidepressant treatment may not be identified by using overlapping markers. These stage-specific regional effects may partly explain the discrepancies observed in the literature regarding the regional effects of animal models of depression and antidepressants on neurogenesis.

Given its interactions with the HPA axis and the limbic system, it has been suggested that the temporal hippocampus might be more vulnerable to the effects of stress and preferentially involved in the effects of antidepressants. Our results indicate otherwise and suggest that neurogenesis in both septal and temporal divisions of the hippocampus might be associated to stress-related disorders and to the effects of antidepressants. Indeed, in our study, UCMS decreased proliferation, survival and the number of immature neurons uniformly along the septo-temporal axis, and most of these effects were reversed by fluoxetine treatment in both septal and temporal divisions.

Additionally, EE is a behavioral manipulation known to have antidepressant and anxiolytic properties (Benaroya-Milshtein et al., 2004; Fox et al., 2006; Laviola et al., 2008; Brenes et al., 2009) and importantly stimulated proliferation, survival of newborn cells and the number of immature neurons specifically in the septal divisions of the hippocampus. As it was suggested that both fluoxetine (Santarelli et al., 2003; Airan et al., 2007; Surget et al., 2008, 2011; David et al., 2009) and enrichment (Schloesser et al., 2010) may exert their mood improving effects by recruiting hippocampal newborn neurons, neurogenesis in both septal and temporal divisions may therefore contribute to some extent to these effects, possibly by enhancing distinct hippocampal functions. Others have also highlighted an indirect link between neurogenesis in the septal hippocampus in depressive-like behavior or antidepressant treatment. Learned helplessness, which is a known model of depression, was found to impair cell proliferation and survival specifically in the most anterior third of the hippocampus (Ho and Wang, 2010).

Changes in the expression of neurotrophic factors, which seem implicated in the pathophysiology of depression, the action of antidepressants and the regulation of neurogenesis (Schmidt and Duman, 2007), have also been reported in both septal and temporal divisions of the hippocampus in different models of depression and following antidepressant treatment (Faure et al., 2007; Marais et al., 2008; Soumier et al., 2009; Larsen et al., 2010). Moreover, knockdown of BNDF specifically in the dorsal dentate gyrus but also in the ventral subiculum both lead to depressive-like behavior (Taliaz et al., 2010). It seems therefore that the relation between region-specific changes in neurogenesis, neurotrophic factors, and depressive-like behavior is rather more complex and that both septal and temporal divisions may contribute to the pathophysiology of depression and the effects of antidepressants.

Interestingly in our experiment, while fluoxetine reversed the UCMS-induced effects in both septal and temporal divisions, chronic fluoxetine treatment only affected proliferation and survival in the temporal divisions in control mice. Differential effects of fluoxetine between control animals and challenged animals have already been reported (David et al., 2009; Rainer et al., 2011; Nollet et al., 2012). These state-dependent effects as well as the clear dissociation between the effects of fluoxetine in control mice and the effects of EE suggest that, rather than one mechanism, multiple factors could contribute to the regulation of neurogenesis but in a region-specific manner. While it is only speculative, regional differences in monoaminergic, glutamatergic and GABAergic transmission (Gage and Thompson, 1980; Jinno and Kosaka, 2006, 2010) as well as in the expression or function of various 5-HT receptors, such as 5-HT1A, 5-HT2C, or 5-HT2B (Tanaka et al., 2012) which have been linked to the pro-neurogenic effects of antidepressants (Santarelli et al., 2003; Banasr et al., 2004; Soumier et al., 2009; Klempin et al., 2010; Diaz et al., 2012) may likely account for some of those differences. Among other factors, the detrimental and pro-neurogenic effects of chronic stress, antidepressants, and enrichment/exercise have also all been linked to glucocorticoids (Montaron et al., 2003; Wong and Herbert, 2006; Oomen et al., 2007; Anacker et al., 2011; Lehmann et al., 2013). Topographical differences in glucocorticoid receptors regulation and function (Robertson et al., 2005; Maggio and Segal, 2009; Lin et al., 2012) may therefore possibly account for those results.

## CONCLUSIONS

In this study we show that UCMS, fluoxetine treatment, and environmental enrichment have distinct region-specific effects on neurogenesis. In view that stimulation of newborn neurons is thought to be important for the behavioral effects of both antidepressant treatment and environmental enrichment, and given the functional heterogeneity of the hippocampus this could be of particular relevance. While there is still much debate regarding the implication of neurogenesis in stress-related and mood disorders (Petrik et al., 2012), suppression of neurogenesis has been shown to impair hippocampal functions underlain by both septal and temporal divisions of the hippocampus, such as spatial learning (Goodman et al., 2010), discrimination of overlapping contexts (Sahay et al., 2011) cognitive flexibility (Burghardt et al., 2012), termination of the stress response (Schloesser et al., 2009; Snyder et al., 2011; Surget et al., 2011) or anxious behaviors (Revest et al., 2009). Regional changes in neurogenesis could potentially correlate to specific functional changes and contribute differently to the pathophysiology of stress-induced affective disorders and cognition. Supporting this view we show that UCMS did not affect the same newborn cells populations in those two areas. While the functional significance of such stage-specific effects is not clear, differential properties of newborn neurons in septal and temporal divisions may confer them distinct and complementary abilities to modulate hippocampal network activity (Airan et al., 2007; Lacefield et al., 2012) and contribute to hippocampal functions. A better understanding of the mechanisms involved in such differential region-specific regulation of neurogenesis may be a useful approach to study the role of newborn neurons. It is also likely that, given the molecular heterogeneity of the hippocampus, different mood-improving drugs could have distinct regional effects on neurogenesis depending on their initial target. Deciphering how regional changes in neurogenesis along the septo-temporal axis can affect behavior will provide incremental insight regarding the participation of newborn neurons in the action of antidepressants.

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